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Original Article

Influence of temperature and growth regulators on wound periderm formation of potato tuber

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Abstract: When a potato tuber is wounded, cork cambium is formed just beneath the wounded area. The cambium produces waterproofing polymeric and polyphenolic compounds known as suberin that are deposited on the cell wall of the tissue. Little is known about the ideal conditions and endogenous factor(s) that control the formation of suberized area, also called wound periderm (WP). In this study, we observed that WP formation is largely dependent on temperature. Among different temperatures (15°C - 35°C) tested, only 25°C promoted WP formation. Washing of the tissue discs immediately after cutting severely inhibited WP formation. However, keeping the discs for 4 days or more followed by washing failed to show any inhibitory effects. Finally, the washed tissue discs were treated with different plant hormones and among them, abscisic acid showed a stimulatory effect in inducing WP formation.

Keywords: Potato tuber, Wound Periderm, Suberization, Plant hormone.

1. Introduction

Post harvest deterioration of tubers is a serious problem and adversely affects the quality of the tuber. Wounding of potato tubers during harvesting and post harvest handling can increase water loss and infection by microorganisms. When potato tubers are wounded, they form a protective layer next to the exposed surface [1]. The protective layer is termed as wound periderm (WP). WP is produced by the activity of cork-cambium (phellogen), a kind of secondary meristem that is formed immediately after the wounding. On the cell wall of this cork-tissue, suberin that consists of phenolic and aliphatic compounds is accumulated and forms a strong barrier both to the diffusion of water and to the invasion of pathogens. Suberization develops a distinct polyaliphatic layer with associated phenolic acids typically deposit between the primary cell wall and the plasma membrane prior to the secondary wall formation [2,3,4]. This multilayered aliphatic polymer with its associated waxes is thought to provide suberized tissue with their water repellent [5, 6].

The temperature limits for wound responses have not yet been established for potato tubers. In case of

potatoes, a temperature that favors maximum suberization and periderm formation can also promote tuber ageing, sprouting and most importantly, the growth of microorganisms that may cause decay [7]. Relative humidity is reported to be important but less critical than temperature in the wound healing process. With low humidity desiccation of the wound surface takes place that inhibits suberization. In contrast, saturated atmospheres may hamper WP development by inducing cell proliferation at the wound surface [1]. Various hormones seem to be an important intermediate in the signal transduction pathway leading to the induction of suberin [8]. Since suberization appears to be triggered by wounding, it is likely that the hormonal compounds that lead to WP formation are generated soon after the wounding. Experiment with potato tubers have shown that suberization can be inhibited by washing the wound with water. Various cytokines are removed by these washes and exogenous cytokines applied to wash tissues partially restore suberin production [8]. The present investigation was undertaken using potato plants as material in order to obtain further information on the suberization and WP

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formation of tissue discs under certain experimental condition.

Understanding of the basis for wound responses in potato tuber could lead to innovative control measure for potato diseases and dehydration during storage. These important post harvest processes will provide producers and consumers more benign and effective means to improve the storability and nutritional quality of this important crop.

2. Materials and Methods

2.1 Preparations of tissue discs from potato tuber

Tissue discs were prepared from the medullary tissue of potato tuber (*Solanum tuberosum* L. CV. Irish Cobbler) using a cork borer (2.0cm in diameter and 5.0mm in thickness with a fresh weight of approximately 2.0-2.5gm). For incubation, tissue discs were kept within a petri dish that contained a small dish filled with water to provide humid conditions. Petri dishes were then incubated at various temperatures in the dark for 7 days to induce WP formation.

2.2 Measurement of WP formation

Since the WP act as a strong barrier to water diffusion, the resistance of the tissue surface to water diffusion is a good measurement of degree of WP formation. At first, for this purpose, we measured the water potential of the tissue discs. But the procedure was very time consuming and the result obtained had a large variation. So we decided to measure water loss from the tissue discs during the incubation in open space. After the 7 days-incubation in a moist petri dish, the discs were transferred to open space at 25°C (relative humidity, ca. 30%) and time course changes in fresh weight was measured. The percent of the initial water content of potato tissue discs was calculated from the following formula-

$$WC = \frac{\text{water content of a tissue disc}}{\text{Initial water content of tissue disc}} \times 100$$

Where, water content of a tissue was calculated from the subtraction between the fresh weight at different day intervals and dry weight of the tissue disc. Dry weight was measured after drying the tissue discs at 95°C overnight. All experiments were repeated at least twice with 20 replicates.

2.3 Effects of plant hormones on WP formation

To examine the effects of various plant hormones on WP formation, 5×10^{-7} mol each of Benzyladenine (BA), Jasmonic acid (JA), Indole-3-acetic acid (IAA), Absciscic acid (ABA) and Gibberellic acid (GA) were applied onto tissue discs after washing. The discs were then incubated at 25°C. To examine the stimulatory effect of ABA on WP formation, different amounts of

ABA (0 , 5.0×10^{-10} , 5.0×10^{-9} , 5.0×10^{-8} , and 5.0×10^{-7} mol) were applied on washed tissue discs.

2.4 Determination of ABA level in tuber

Endogenous ABA was extracted from freshly harvested and old tuber and also from unwashed and washed tissue discs. The aqueous residue obtained from ethanolic extract (EA) at pH 3.0. The EA fraction was then extracted with 3 volumes of 1M NaHCO_3 at pH 8.0. The pH of the resulting NaHCO_3 fraction was adjusted to 3.0 and then extracted with 3 volumes of EA. The EA fraction was evaporated to dryness to get the ABA fraction. It was then redissolved in 3.0ml of 60% methanol and passed through a Sep-Pak C_{18} cartridge (Boreal Laboratories, USA), which had previously been washed with methanol and then with water. The cartridge was eluted with 3.0ml 60% methanol. Elute was then evaporated to dryness and fractionated twice by HPLC. The HPLC analysis was carried out as follows: column, Nova-Pak C_{18} (LabX, USA) size, 8.0mm X 100mm; solvent, 60% methanol that contained 0.1% acetic acid; flow rate, $1\text{ml}/\text{min}^{-1}$. The elution profile was monitored by absorbance at 260nm. The retention time under these conditions of authentic ABA was 20.08 min. and the fraction of elute corresponding to ABA was collected. The fraction was rechromatographed on the same column in 25% acetonitrile that contained 0.1% acetic acid.

2.5 Histological analysis

Sections (150μ in thickness) were made by the rotary Microslicer (D.T.K-1000, JAPAN) equipped with a razor blade for histological analysis, these sections were observed under microscope (Nikon, type-120, Japan). Polyphenolic compounds emit fluorescence under UV radiation. The degree of suberization was observed by fluorescent microscope.

3. Results and Discussion

3.1 Effects of temperature on WP formation

The rate and extent of WP formation and suberization are largely dependent on temperature. Firstly, we investigated the effect of temperature on the WP formation of wounded potato tuber. Tissue discs kept at 25°C formed considerable WP layer, however, other temperatures (15°C-35°C) almost failed to form WP, and the discs were completely dried within 20 days (Fig. 1). This result suggested that the enzymes/compounds involved in the process of WP formation have optimal activity at 25°C. Incubation of potato discs at 25°C promotes formation of suberin and WP that can effectively reduce weight loss during subsequent storage. On the other hand, the higher temperatures that are required for wound healing also increase the vapor pressure deficit (VPD) of the air around the potatoes. This may help to trigger rapid

suberization and WP formation to prevent weight loss during wound healing.

3.2 Effects of washing

To determine the effect and optimal time of washing, tissue discs were washed for different time under running tap water immediately after the preparation of discs. As shown in Fig. 2, washing for 3 to 6 hrs was enough to cause complete loss of WP forming ability. Well-developed WP and suberization were observed under microscopic examination in unwashed tissue discs whereas slight suberization and WP formation were observed in washed one (data not shown). This result suggested that some factor(s) were produced at the wound surface that might be responsible for WP formation. An attempt was then made to know when and how long this factor(s) were involved to induce WP formation.

The discs were washed for 3 hours at different day intervals after preparation of the discs. When the discs were washed within 3 days after preparation, the WP formation was severely inhibited. By contrast, when the discs were kept in a moist chamber for more than 4 days followed by washing, WP formation was completely unaffected (Fig. 3). Thus, all these indicated that WP inducing factors (WPFs), responsible for the induction of the enzymes involved in the biosynthesis

of suberin components and cell division can be removed by washing the wounded area any time prior to the induction.

3.3 Effects of plant hormones on WP formation

Plant hormones play a key role in the signal transduction pathway that leads to the potato tuber development [8, 9]. So there is a possibility that any of the WFPs may be one of the plant hormones that is responsible for WP formation. To examine this possibility different plant hormones (BA, JA, IAA, ABA and GA) were applied on tissue discs after washing. The tissue discs treated with ABA had a minimum weight loss and showed well developed periderm layer as compared to the discs treated with other hormones (Fig. 4 and 5). However, the amount of ABA applied and the resulting concentration of ABA inside the tissue discs were relatively high. Therefore, it was examined whether or not ABA at low concentrations could induce WP formation. We found that relatively higher concentrations of ABA (5.0×10^{-8} , 5.0×10^{-7} M) showed the better stimulatory effect than lower concentration (5.0×10^{-10} M) (Fig. 6), depicting a concentration dependent stimulatory effect of ABA. The concentration is nearly equal to that of the endogenous ABA of the tissue discs (2.0×10^{-7} M), as described in the next section.

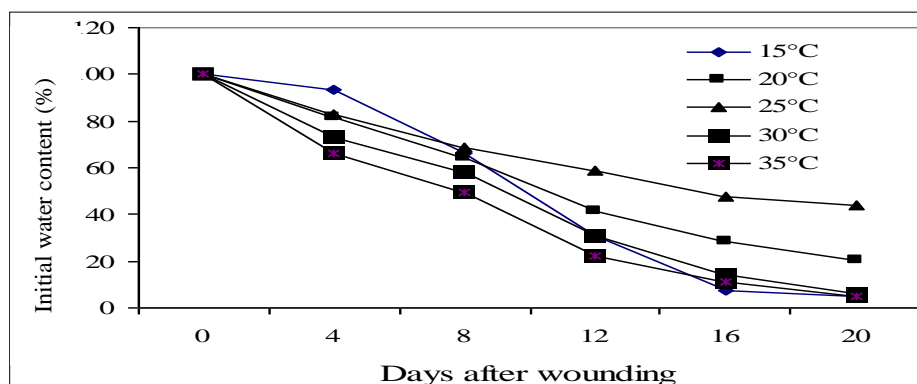


Fig. 1. Effects of different temperature of Wound Periderm (WP) formation. Tissue discs kept at temperature other than 25°C almost failed to form WP and was completely dry within 20 days.

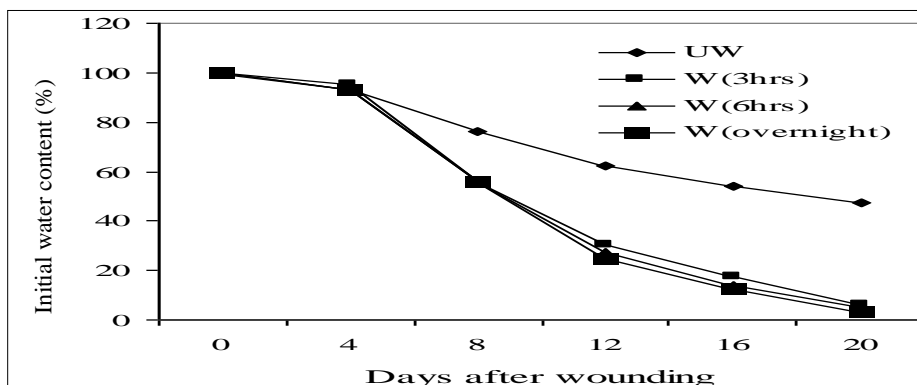


Fig. 2. Weight loss of wound discs as affected by continuous washing. Tissue discs washed for 3 to 6 hrs was enough to cause complete loss of WP forming ability whereas, well-developed WP was observed in unwashed tissue discs (W-Washed; UW Unwashed control).

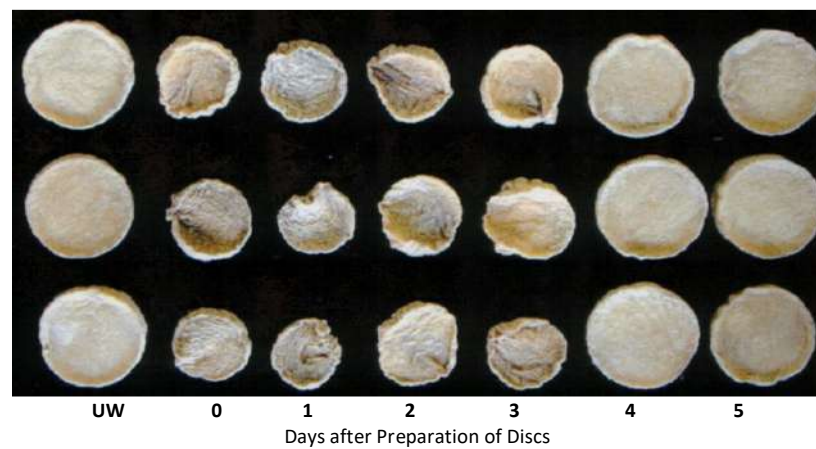


Fig. 3. The effects of washing at different days after preparation of discs on WP formation. WP formation was inhibited when the discs were washed within 3 days after preparation. But, WP formation unaffected when the discs were washed after 4 days of preparation (UW-unwashed control).

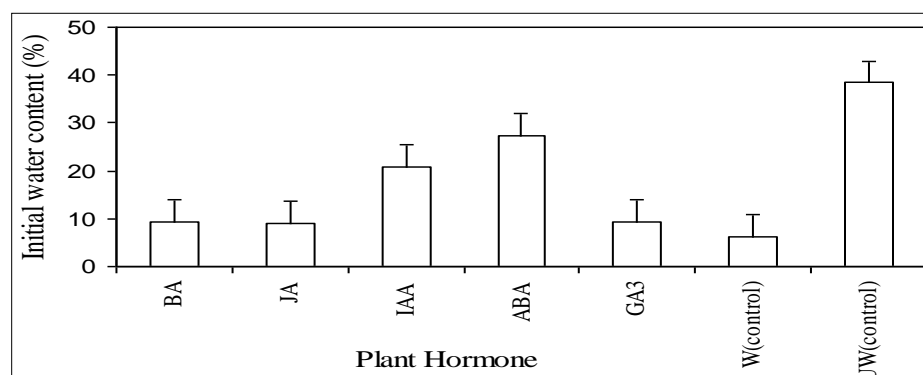


Fig. 4. Effect of various plant hormones on WP formation. Bars represent \pm SE (n=20). Among the plant hormones tested, ABA showed a comparatively better effect on WP formation and suberization process but it couldn't fully restore WP forming ability when compared to unwashed control discs (BA- Benzyladenine; JA- Jasmonic acid; IAA- Indole-3-acetic acid; ABA- Absciscic acid; GA- Gibberellic acid 3; W-Washed control and UW- Unwashed control).

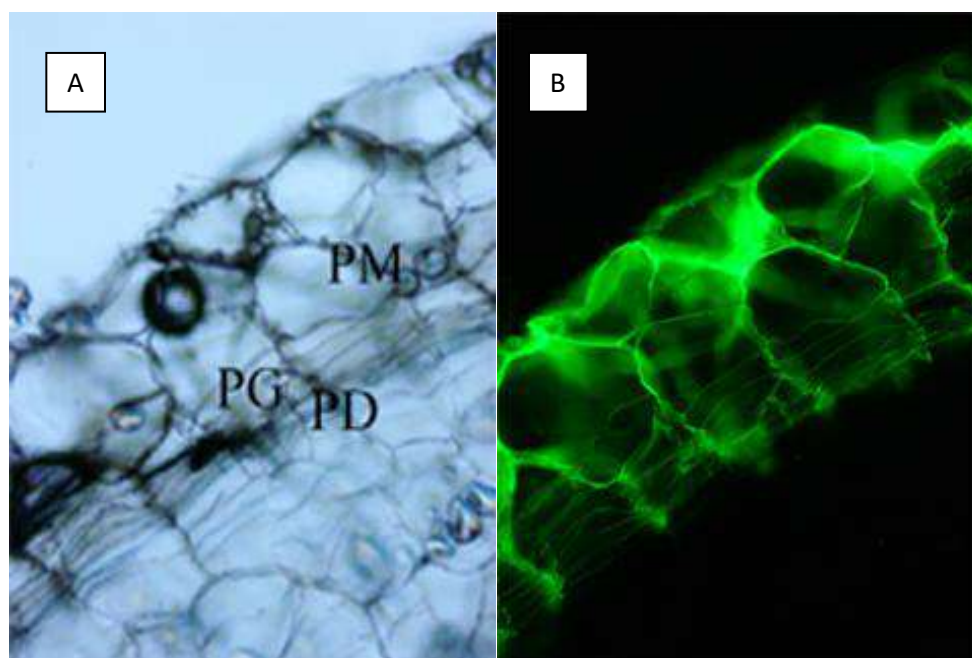


Fig. 5. Light micrographs of periderm section viewed under bright field (A) B. UV auto-fluorescence (B). Washed tissue discs treated with ABA 5×10^{-7} mol. Showed well developed WP and suberized layer (PM- Phellem; PG- Phellogen; PD- Phelloderm).

3.4 ABA levels in potato tissue

The complete loss of WP forming ability was brought about by thorough washing and their restoration by supplying exogenous ABA to the washed tissue discs suggested that ABA might be one of the WPF and that was removed by washing. To precisely examine the involvement of ABA, endogenous ABA levels were compared before and after washing the tissue discs. The level of ABA in unwashed potato tissues was found 55.3ng/g of fresh weight and it was decreased to 8.4ng of ABA/g and 0.39ng of ABA/g, after 3 and 6 hrs washing respectively. In our experiment, we found that WP is forming ability of potato tuber decreasing with the duration of the storage period (data not shown). So, if the ABA is one of the WPF, the level of the ABA must decrease with duration of the storage period. Therefore, the endogenous levels

of ABA in freshly harvested tubers and that of old tubers were compared. We found that fresh tuber contained 55.3ng (2.0×10^{-7} M) whereas old tubers (10 months after washing) contained only 1.8ng of ABA/g of fresh weight (7.0×10^{-10} M). All these results described above indicated that the changes of endogenous level were coinciding with the changes of WP forming ability.

Although, ABA stimulated WP formation and suberization process it couldn't fully restore WP forming ability of tissue discs when compared to unwashed control discs. The result suggested that the presence of any other factor(s) that might act synergistically with ABA to induce WP formation. Further investigation is required to find out the factors other than ABA responsible for WP formation.

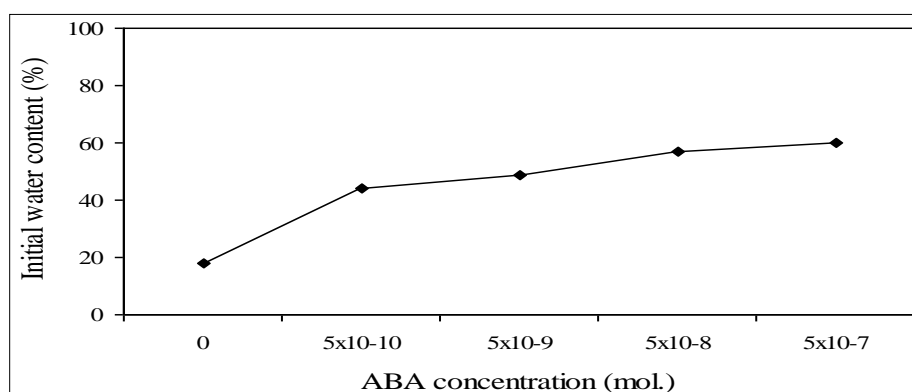


Fig. 6. Effects of various concentrations of ABA on WP formation. Unwashed (control) considered as 100%. Relatively higher concentrations of ABA showed a better stimulatory effect on the WP formation than lower concentration, depicting a concentration dependent stimulatory effect of ABA.

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